

What is claimed:

1. A method of producing a panto-compound comprising culturing a microorganism which overexpresses at least one *Bacillus* pantothenate biosynthetic enzyme under conditions such that the panto-compound is produced.
2. The method of claim 1, wherein the microorganism overexpresses at least one *Bacillus subtilis* pantothenate biosynthetic enzyme.
3. The method of claim 1 or 2, wherein the pantothenate biosynthetic enzyme is selected from the group consisting of ketopantoate hydroxymethyltransferase, pantothenate synthetase, aspartate- $\alpha$ -decarboxylase and ketopantoate reductase.
4. The method of any one of claims 1 to 3, wherein the microorganism overexpresses at least two pantothenate biosynthetic enzymes.
5. The method of any one of claims 1 to 3, wherein the microorganism overexpresses at least three pantothenate biosynthetic enzymes.
6. The method of any one of claims 1 to 5, wherein the panto-compound is selected from the group consisting of pantothenate, pantoate, ketopantoate and  $\beta$ -alanine.
7. A method of producing a panto-compound comprising culturing a ketopantoate reductase-overexpressing (KPAR-O) microorganism under conditions such that the panto-compound is produced.
8. The method of claim 7, wherein the panto-compound is pantothenate or pantoate.
9. The method of claim 7 or 8, wherein the ketopantoate reductase is bacterial-derived.
10. The method of claim 7 or 8, wherein the ketopantoate reductase is derived from *Bacillus*.



11. The method of claim 7 or 8, wherein the ketopantoate reductase is derived from *Bacillus subtilis*.

12. The method of any one of claims 7 to 11, wherein the KPAR-O microorganism further overexpresses at least one pantothenate biosynthetic enzyme in addition to overexpressing ketopantoate reductase.

13. The method of claim 12, wherein the KPAR-O microorganism further overexpresses at least one of ketopantoate hydroxymethyltransferase, pantothenate synthetase and aspartate- $\alpha$ -decarboxylase.

14. A method of producing pantothenate in a manner independent of precursor feed comprising culturing an aspartate- $\alpha$ -decarboxylase-overexpressing (A $\alpha$ D-O) microorganism having a deregulated isoleucine-valine (*ilv*) pathway under conditions such that pantothenate is produced.

15. A method of producing at least 2 g/L pantothenate in a manner independent of aspartate or  $\beta$ -alanine feed comprising culturing an aspartate- $\alpha$ -decarboxylase-overexpressing (A $\alpha$ D-O) microorganism under conditions such that pantothenate is produced.

16. A method of producing at least 2 g/L pantothenate in a manner independent of valine or  $\alpha$ -ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.

17. A method of producing at least 30 g/L pantothenate in a manner independent of aspartate or  $\beta$ -alanine feed comprising culturing an aspartate- $\alpha$ -decarboxylase-overexpressing (A $\alpha$ D-O) microorganism under conditions such that pantothenate is produced.

18. A method of producing at least 30 g/L pantothenate in a manner independent of valine or  $\alpha$ -ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.



19. A  $\beta$ -alanine independent high yield production method for producing pantothenate comprising culturing a manipulated microorganism under conditions such that pantothenate is produced at a significantly high yield.

5 20. The method of any one of claims 14 to 19, wherein the microorganism overexpresses acetohydroxyacid synthetase or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* sequence.

21. The method of any one of claims 14 to 19, wherein the  
10 microorganism overexpresses acetohydroxyacid isomeroreductase or is transformed with a vector comprising an *ilvC* nucleic acid sequence.

22. The method of any one of claims 14 to 19, wherein the  
15 microorganism overexpresses dihydroxyacid dehydratase or is transformed with a vector comprising an *ilvD* nucleic acid sequence.

23. The method of any one of claims 19 to 22, wherein the  
microorganism overexpresses aspartate- $\alpha$ -decarboxylase or is transformed with a vector  
comprising a *panD* nucleic acid sequence.

24. The method of any one of claims 14 to 23, wherein the  
microorganism further has a deregulated pantothenate biosynthetic pathway.

25. The method of any one of claims 14 to 24, wherein the  
25 microorganism further has at least one mutant gene selected from the group consisting of a mutant *avlA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

26. The method of claim 24, wherein the microorganism  
overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase,  
30 pantothenate synthetase and aspartate- $\alpha$ -decarboxylase.

27. The method of claim 24 or 26, wherein the microorganism is  
transformed with a vector comprising a *panBCD* nucleic acid sequence or a vector  
comprising a *panEI* nucleic acid sequence.



28. The method of any one of claims 14 to 16 and 19 to 27, wherein pantothenate is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

5 29. The method of claim 20, wherein the microorganism overexpresses acetohydroxyacid synthetase derived from *Bacillus* or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* nucleic acid sequence derived from *Bacillus*.

10 30. The method of claim 21, wherein the microorganism overexpresses acetohydroxyacid isomeroreductase derived from *Bacillus* or is transformed with a vector comprising an *ilvC* nucleic acid sequence derived from *Bacillus*.

15 31. The method of claim 22, wherein the microorganism overexpresses dihydroxyacid dehydratase derived from *Bacillus* or is transformed with a vector comprising an *ilvD* nucleic acid sequence derived from *Bacillus*.

20 32. The method of claim 23, wherein the microorganism overexpresses aspartate- $\alpha$ -decarboxylase derived from *Bacillus* or is transformed with a vector comprising a *panD* nucleic acid sequence derived from *Bacillus*.

25 ~~33.~~ The method of claim 24 or 26, wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate- $\alpha$ -decarboxylase derived from *Bacillus*.

34. The method of claim 27, wherein the vector comprises a *panBCD* nucleic acid sequence or a *panEI* nucleic acid sequence derived from *Bacillus*.

30 35. A method of producing a panto-compound comprising contacting a composition comprising at least one pantothenate biosynthesis pathway precursor or isoleucine-valine biosynthesis pathway precursor with at least one isolated *Bacillus* enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate- $\alpha$ -decarboxylase, under  
35 conditions such that the panto-compound is produced.



36. A method of producing  $\beta$ -alanine comprising culturing an aspartate- $\alpha$ -decarboxylase-overexpressing (AaD-O) microorganism under conditions such that  $\beta$ -alanine is produced.

5 37. The method of claim 36, wherein the AaD-O microorganism has a mutation in a nucleic acid sequence encoding a pantothenate biosynthetic enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase and pantothenate synthetase.

10 38. A method of producing  $\beta$ -alanine comprising contacting a composition comprising aspartate with an isolated *Bacillus* aspartate- $\alpha$ -decarboxylase enzyme under conditions such that  $\beta$ -alanine is produced.

15 39. A method for enhancing production of a panto-compound comprising culturing a mutant microorganism having a mutant *coaX* gene under conditions such that the panto-compound production is enhanced.

20 40. The method of claim 39, wherein said recombinant microorganism has a mutant *coaA* gene.

25 41. A method of producing a panto-compound comprising a pantothenate kinase mutant microorganism under conditions such that the panto-compound is produced at a significantly high yield.

42. The method of claim 41, wherein said mutant microorganism has a mutant *coaA* gene.

43. The method of claim 41, wherein said mutant microorganism has a mutant *coaX* gene.

30 44. The method of claim 41, where said mutant microorganism has a mutant *coaA* and *coaX* gene.

35 45. The method of any one of claims 39 to 44, wherein said panto-compound is selected from the group consisting of ketopantoate, pantoate or pantothenate.



46. The method of any one of claims 39 to 44, wherein said panto-compound is pantothenate.

47. The method of any one of claims 39 to 44, wherein said panto-compound is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

48. The method of any one of claims 39 to 44, wherein said recombinant microorganism further has a deregulated pantothenate biosynthetic pathway or further has a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

49. The method of claim any one of claims 39 to 44, wherein said recombinant microorganism further overexpresses *panD* and *panE*.

50. The method of any one of claims 39 to 44, wherein said recombinant microorganism further has at least one mutant gene selected from the group consisting of a mutant *avtA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

51. A method for enhancing production of a panto-compound comprising culturing a microorganism that has a deregulated pantothenate biosynthetic pathway and that also has a mutation that results in reduced pantothenate kinase activity under conditions such that the panto-compound production is enhanced.

52. A method for identifying compounds which modulate pantothenate kinase activity comprising contacting a recombinant cell expressing pantothenate kinase encoded by the *coaX* gene with a test compound and determining the ability of the test compound to modulate pantothenate kinase activity in said cell.

53. The method of claim 52, wherein said cell further comprises a mutant *coaA* gene encoding a pantothenate kinase having reduced activity.

54. The method of any one of claims 1 to 51, wherein the microorganism is Gram positive.

55. The method of any one of claims 1 to 51, wherein the microorganism is Gram negative.



56. The method of any one of claims 1 to 51, wherein the microorganism is a microorganism belonging to a genus selected from the group consisting of *Bacillus*, *Corynebacterium*, *Lactobacillus*, *Lactococci* and *Streptomyces*.

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57. The method of any one of claims 1 to 51 and 54 to 56, wherein the microorganism is of the genus *Bacillus*.

58. The method of any one of claims 1 to 51 and 54 to 57, wherein the microorganism is *Bacillus subtilis*.

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59. The method of any one of claims 1 to 13, 35, 39 to 51 and 54 to 58, further comprising recovering the panto-compound.

60. The method of any one of claims 14 to 34 and 54 to 58, further comprising recovering the pantothenate.

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61. The method of any one of claims 1 to 14, 35, 39 to 46, 48 to 51 and 54 to 59, wherein the panto-compound is produced at a level greater than 2 g/L.

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62. A recombinant microorganism which overexpresses at least one *Bacillus* pantothenate biosynthetic enzyme.

63. The recombinant microorganism of claim 62, which overexpresses at least one *Bacillus subtilis* pantothenate biosynthetic enzyme.

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64. The recombinant microorganism of claim 62 or 63, wherein the pantothenate biosynthetic enzyme is selected from the group consisting of ketopantoate hydroxymethyltransferase, pantothenate synthetase, aspartate- $\alpha$ -decarboxylase and ketopantoate reductase.

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65. The recombinant microorganism of any one of claims 62 to 64, wherein the pantothenate biosynthetic enzyme is ketopantoate reductase.

66. A recombinant microorganism which overexpresses aspartate- $\alpha$ -decarboxylase and has a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

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67. A recombinant microorganism having a mutant *coaX* gene, said mutant *coaX* gene encoding reduced pantothenate kinase activity in said microorganism.

68. The recombinant microorganism of claim 67 further having a mutant *coaA* gene, said mutant *coaA* gene encoding reduced pantothenate kinase activity in said microorganism.

69. A recombinant microorganism having a mutant *coaX* gene and optionally having a mutant *coaA* gene, said mutant microorganism having reduced pantothenate kinase activity as compared to a microorganism having wild-type *coaA* and *coaX* genes.

70. A recombinant microorganism comprising a vector comprising an isolated *coaX* gene.

71. A recombinant microorganism that overproduces a panto-compound, the microorganism having a deregulated pantothenate biosynthetic pathway and having at least one mutation that results in a decrease in the capacity of the microorganism to synthesize Coenzyme A (CoA).

72. The recombinant microorganism of claim 71, having at least one mutation that results in a reduced level of pantothenate kinase activity.

73. The recombinant microorganism of claim 72, having a mutation in a *coaA* gene, or homologue thereof, that results in a reduced level of CoaA enzyme activity.

74. The recombinant microorganism of claim 72, having a mutation in a *coaX* gene, or homologue thereof, that results in a reduced level of CoaX enzyme activity.

75. The recombinant microorganism of claim 72, having a mutation in a *coaA* gene, or homologue thereof, and having a mutation in a *coaX* gene, or homologue thereof, the mutations resulting in reduced levels of CoaA enzyme activity and reduced CoaX enzyme activity.



76. The recombinant microorganism of any one of claims 66 to 70 which further has a deregulated pantothenate biosynthetic pathway.

77. The recombinant microorganism of any one of claims 62 to 65  
5 and 67 to 75, further having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

78. The recombinant microorganism of any one of claims 62 to 77, which is Gram positive.

10 79. The recombinant microorganism of claim 78 belonging to a genus selected from the group consisting of *Bacillus*, *Corynebacterium*, *Lactobacillus*, *Lactococci* and *Streptomyces*.

15 80. The recombinant microorganism of claim 79 belonging to the genus *Bacillus*.

81. The recombinant microorganism of claim 80 which is *Bacillus subtilis*.

20 82. A recombinant microorganism selected from the group consisting of PA221, PA235, PA236, PA313, PA410, PA402, PA403, PA411, PA412, PA413, PA303, PA327, PA328, PA401, PA340, PA342, PA404, PA405, PA374, PA354, PA365, PA377, PA651 and PA824.

25 83. A recombinant vector for use in the production of panto-compounds comprising a nucleic acid sequence which encodes at least one *Bacillus* pantothenate biosynthetic enzyme operably linked to regulatory sequences.

30 84. The vector of claim 83, comprising a nucleic acid sequence which encodes at least one *Bacillus subtilis* pantothenate biosynthetic enzyme.

35 85. The vector of claim 84, wherein the nucleic acid sequence encodes at least one of ketopantoate hydroxymethyltransferase, pantothenate synthetase, aspartate- $\alpha$ -decarboxylase and ketopantoate reductase.



86. A recombinant vector comprising at least one nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29 and SEQ ID NO:59.

5 87. The vector of claim 84, wherein the nucleic acid sequence encodes ketopantoate reductase.

10 88. A vector comprising a mutant *coaX* gene, said mutant encoding a pantothenate kinase enzyme having reduced activity.

89. A vector comprising an isolated *coaX* gene.

90. A vector comprising an isolated *Bacillus coaX* gene.

15 91. A vector comprising an isolated *Bacillus subtilis coaX* gene.

92. The vector of any one of claims 86 and 89 to 91, which further comprises regulatory sequences.

20 93. The vector of any one of claims 83 to 85, 87 and 92, wherein the regulatory sequences comprise a constitutively active promoter.

25 94. The vector of claim 93, wherein the constitutively active promoter comprises  $P_{veg}$  (SEQ ID NO:41),  $P_{15}$  (SEQ ID NO:39) or  $P_{26}$  (SEQ ID NO:40) sequences.

95. The vector of claim 83, wherein the regulatory sequences comprise at least one artificial ribosome binding site (RBS).

30 96. The vector of claim 95, wherein the artificial RBS comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56 and SEQ ID NO:57.

35 97. A vector selected from the group consisting of pAN004, pAN005, pAN006, pAN236, pAN423, pAN428, pAN429, pAN441, pAN442, pAN443, pAN251,



~~pAN267~~, pAN256, pAN257, pAN263, pAN240, pAN294, pAN296, pAN336, pAN341  
and pAN342.

98. A recombinant microorganism comprising the vector of claim 86  
5 or 93.

99. An isolated nucleic acid molecule which encodes at least one  
*Bacillus* pantothenate biosynthetic gene.

100. The isolated nucleic acid molecule of claim 99 which encodes at  
10 least one *Bacillus subtilis* pantothenate biosynthetic gene.

101. The isolated nucleic acid molecule of claim 99 or 100 which  
encodes ketopantoate reductase.

102. An isolated *Bacillus* pantothenate biosynthetic enzyme  
15 polypeptide.

103. An isolated *Bacillus subtilis* pantothenate biosynthetic enzyme  
20 polypeptide.

104. An isolated *Bacillus* ketopantoate reductase polypeptide.

105. An isolated *Bacillus subtilis* ketopantoate reductase polypeptide.

106. An isolated *Bacillus* aspartate- $\alpha$ -decarboxylase polypeptide.

107. An isolated *Bacillus subtilis* aspartate- $\alpha$ -decarboxylase  
polypeptide.

108. An isolated nucleic acid molecule comprising a mutant *coaX*  
30 gene.

109. An isolated nucleic acid molecule comprising a *coaX* gene.

110. An isolated pantothenate kinase protein encoded by a *coaX* gene.